

Growth and survival of *Diphyllobothrium erinacei* in bileless dogs

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It has been recognized that bile plays an important role in determining host specificity of intestinal parasites (Smyth 1962; Smyth & Haslewood, 1963). However, little is known of the possible function of bile on the growth and survival of cestodes in the final host. Except for the works of some investigators, such as on the depressing growth of *Hymenolepis diminuta* in bileless rats (Goodchild, 1958, 1960), and on the successful cultivation of *Spirometra mansonioides* (Berntzen & Mueller, 1964) and *Taenia crassiceps* (Taylor, 1963) *in vitro*, the problems on this point have received a little attention, and additional works are expected.

The present study was undertaken to know the responsibility of bile for the growth of plerocercoids and for the survival of adult *Diphyllobothrium erinacei* in the final host (dog). Surgical biliary obstruction was applied as a possible means, and details are reported in this paper.

Materials and Methods

Plerocercoids were obtained from snakes (*Elaphe quadrivirgata*) or from mice which had been thriven with plerocercoids 40 to 60 days before use.

Both sexes of mongrel dogs, weighing 7 to 11 kg, were used as host. In order to prevent bile from mixing with the contents of intestine of dogs, the common bile duct was ligated and separated at the intramural portion under pentobarbital (Nembutal) anesthesia (Kimoto, 1967). After operation,

animals (bileless dogs) were kept in the isolated cages and were given dog food in pellet form (Oriental) once daily and water *ad libitum*. The fecal examination on each dog was performed by means of both thin smear and brine floatation methods.

At autopsy, worms recovered from the intestine of dogs were transferred to physiological salt solution to survey their activity, and were subsequently fixed in 10% formalin stained with Mayer's borax carmine for microscopical examination.

Results

All of bile duct-ligated dogs had a slightly poor appetite, became dullness and developed jaundice.

At autopsy, viscera was yellowish in colour, and both gall bladder and ligated bile duct distended and congested with approximately 30 to 50 ml of thickened bile.

Experiment 1. Growth of plerocercoids in bileless dogs.

Results obtained are shown in Table 1.

Ten out of 12 dogs were ligated their bile duct, and were challenged with 30 plerocercoids on the 5th day after operation. While, 2 intact dogs were also infected with the same number of plerocercoids and were served as control.

Five out of 10 bileless dogs and 1 out of 2 control dogs were sacrificed on the 3rd day after plerocercoid ingestion (Group 1). At autopsy, 1 (3.3%), 1 (3.3%), 8 (26.7%), 10 (33.3%), 13 (43.3%) worms were recovered from the duodenum or from the jejunum of

Table 1 Growth of *D. erinacei* plerocercoids in bileless dogs

Group	Bile duct ligation and No. of dog	Time at autopsy after plerocercoid ingestion (day)	No. of plerocercoid ingested	No. of worms recovered (per cent)
1	Done 5	3	30	1(3.3), 1(3.33), 8(26.7) 10(33.3), 13(43.3)
	Control 1	3	30	29(96.7)
2	Done 5	6	30	0, 0, 0, 0, 0
	Control 1	6	30	28(93.3)

Table 2 Survival of adult *D. erinacei* in bileless dogs

Dog number	Bile duct ligation	No. of plerocercoid ingested	Duration of egg production after operation (day)	No. of worms recovered at autopsy
1	Done	20	4	0
2	Done	20	2	0
3	Done	20	4	0
4	Done	20	5	0
Control	None	20	—	16 (80%)

5 bileless dogs. Average length of the worms was 2.6 mm. All worms had well-developed scolex and the strobila with no characteristic structure. While, in a control dog 29 (96.7%) worms with segment-like furrows on the strobila, 11.4 mm in average length, were found.

The remaining 6 dogs were sacrificed on the 6th day after ingestion (Group 2). At autopsy, no worms were recovered from 5 bileless dogs. While, in a control dog 28 (93.3%) young worms, 187.3 mm in average length, were recovered.

Experiment 2. Survival of previously infected adult worms in bileless dogs.

Result obtained are summarized in Table 2.

Twenty plerocercoids were previously administered to each of 5 dogs. On the 9th, 10th or 11th day after ingestion, eggs of the worm were found in the feces of the dog. On the 3rd day after the beginning of egg production, 4 out of 5 dogs were ligated the bile duct as described above. The eggs in the stools became disappeared on the 2nd, 4th, 4th and 5th day after operation. Two out of 4 dogs were killed

on the 8th day and the remainings on the 10th day after operation. No worms were recovered from these 4 dogs. When a intact dog was sacrificed on the 10th day after ingestion, 16 (80%) adult worms were harbored.

Discussion

The present investigation demonstrated that plerocercoids of *Diphyllbothrium erinacei* could not develop to its mature stage in bileless dogs. A number of plerocercoids were found to survive for 3 days in the intestine of dogs, but no worms were recovered from dogs sacrificed 6 days after ingestion. Adult worms, previously established in the dog, could not maintain their egg production for more than 5 days after biliary obstruction. These worms seemed to die out immediately after operation. Absence of bile exerts, therefore, profound effect upon growth and survival of this species.

Goodchild (1958) indicated that in bileless rats no tapeworms became established on feeding cysticercoids of *Hymenolepis dimi-*

nuta to the host, and growth and sexual maturation of implanted adult worms was atypical. His works have been questioned on the grounds that the worms may be affected by the secondary actions attributable to the absence of bile rather than due to the removal of direct action of bile alone (Rothman, 1959; Smyth & Haslewood, 1963). In fact, bile salts inhibit the metabolism of this worm (Rothman, 1958), and cysticeroids can develop to adult worms *in vitro* in complete absence of bile (Berntzen, 1961; Schiller, 1965). Ginger and Fairbairn (1966) suggested that the absence of bile might remove the essential dietary fatty acids of parasites. With regard to the present work, also, it cannot be doubted that the biliary obstruction upset the normal physiology of the intestine of dogs, and such still undefined abnormalities might have a ill-effect upon growth and survival of the worm.

It is noteworthy that a number of plerocercoids were able to survive at least for 3 days of infection in bileless dogs. The metamorphosis of worms during this survival period seemed to follow the normal pattern of development in an intact dog (Iwata, 1933; Takahashi, 1959a), although the growth of worms in bileless dogs was inferior to that in normal ones. These findings are in conflict with the result reported by Berntzen & Mueller (1964), who described that in *Spirometra mansonioides*, closely related to *D. erinacei*, the first step of growth consists in the permanent eversion of larval scoleces which was able to be produced *in vitro* using "evagination solution", in that the sodium taurocholate or cat bile was to be essential. It is also interesting to note that larval *Taenia crassiceps* survived for a maximum period as they were subjected to enzymic digestion before being cultured *in vitro*, and worms grew in Eagle's medium supplemented with sodium taurocholate were longer than those grew in the other mediums (Taylor, 1963). It is assumed that in *D. erinacei* factors

stimulating the first step of growth other than bile may exist. But because the result obtained from this experiment is not enough to know the direct action of bile that further study must be undertaken before submitting a decided conclusion.

Summary

The possible role of bile of dogs on the growth of the plerocercoid and the adult *Diphyllobothrium erinacei* was investigated. A number of plerocercoids ingested in bileless dogs were found to survive at least for 3 days after ingestion. Development of worms during these periods seemed to correspond to that in an intact host. It is noteworthy that plerocercoids could organize the scoleces even in the bileless environment. The further process of growth might be arrested by the absence of bile and no worms were recovered from dogs sacrificed on the 6th day after ingestion. Adult worms, previously established in the dogs, could not maintain their egg production for more than 5 days after biliary obstruction. The worms seemed to die out soon thereafter. It is concluded that bile would be responsible for the development of plerocercoids and for survival of adult in the dog which is the final host of *D. erinacei*.

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マンソン裂頭条虫の発育と生存におよぼす胆汁の影響

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胆汁あるいは胆汁酸は、寄生虫の発育にともなう形態的、生理的变化に何らかの作用をおよぼしている場合が多く、宿主特異性発現の要因のひとつとして注目されている。条虫類の発育あるいは生存に胆汁が関与していることは、*Hymenolepis diminuta* (Goodchild, 1958), *Spirometra mansonioides* (Berntzen & Mueller, 1964), *Taenia crassiceps* (Taylor, 1963) について知られているが、その作用の本態はまだ究明されていない。本実験は、マンソン裂頭条虫 (*Diphyllobothrium erinacei*) plerocercoid の発育と成条虫の生存に対する、終宿主(イヌ)の胆汁の関与を解明するために行なわれた。

実験1. 総胆管を結紮切断し、胆汁が腸管内に流入するのを阻止したイヌを実験に供した。このイヌに plerocercoid を経口投与したところ、虫体はこの宿主の腸管内で、少なくとも3日間は生存した。その間に吸溝が発達した。しかし、虫体の発育程度、寄生率の点では対照

に比べて劣っていた。plerocercoid を投与後6日目のイヌからは、虫体は見出されなかった。

実験2. あらかじめ、イヌに plerocercoid を経口投与し、このイヌの糞便中に虫卵を検出することによって、虫体が成熟したことを確認した後で、このイヌの総胆管を、前の実験と同様に結紮切断した。虫卵は、術後5日以内にイヌの便中から消失した。また、その後でイヌを剖検したところ、虫体をまったく認めなかった。

すなわち、本条虫の plerocercoid は、胆汁が欠除した腸管内では、成熟しえなかった。また成条虫は、腸管内の胆汁が欠除することによって、数日以内に死滅することがわかった。一方、plerocercoid のあるものが、胆汁が欠除した環境下でも、ある程度発育しうることが、成条虫への発育を誘発する胆汁以外の要因の存在を暗示している。